VERIFICATION OF TRANSLATION

I, Junro Shinoda

of 6th Floor, Yusei Fukushi Kotohira Bldg., 14-1, Toranomon 1-chome, Minato-ku, Tokyo, 105-0001, Japan

declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

2. That the attached document is a true and correct translation made by me to the best of my knowledge and belief of: -

a) The specification of Japanese Patent Application No.114793/2004

November 30, 2009

Junro Shinoda

(Signature of Translator)

(No witness required)

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[Inventor]

[Domicile/residence] 225-903 Yakushicho, Imadegawasagaru, Omiyadori, Kamigyo-ku, Kyoto-shi, Kyoto

[Name] SUGIYAMA Hiroshi

[Inventor]

[Domicile/residence] 2-B Mezon nishikawa, 11 Umenokicho, Itizyouzi, Sakyo-ku, Kyoto-shi, Kyoto

[Name] BANDO Toshikazu

[Applicant]

[Domicile/residence] 21-8, Akasaka 3-chome, Minato-ku, Tokyo

[Name] TMRC Co., Ltd.

[Representative] UENO Takeshi

[Telephone number] 03-3583-3370

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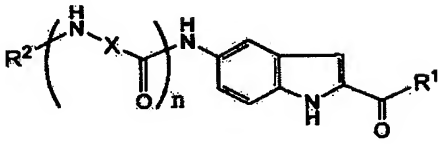
[Document] Claims

[Claim 1]

A hairpin polyamide represented by general formula [1]:

[Chemical formula 1]



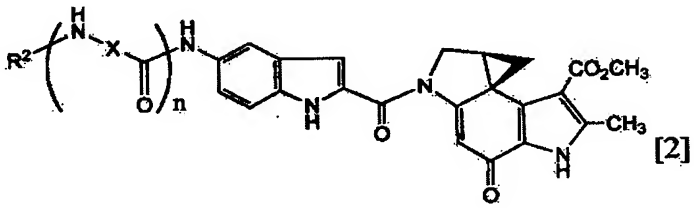
[1]

(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^1 represents an alkylating moiety; R^2 represents a pyrrole-imidazole polyamide compound consisting from a hydrogen atom, a lower alkyl group or an acetyl group.)

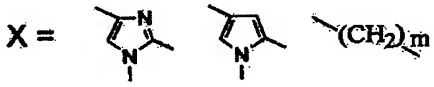
[Claim 2]

The hairpin polyamide according to claim 1, wherein the hairpin polyamide represented by the general formula [1] is represented by general formula [2]:

[Chemical formula 2]



[2]



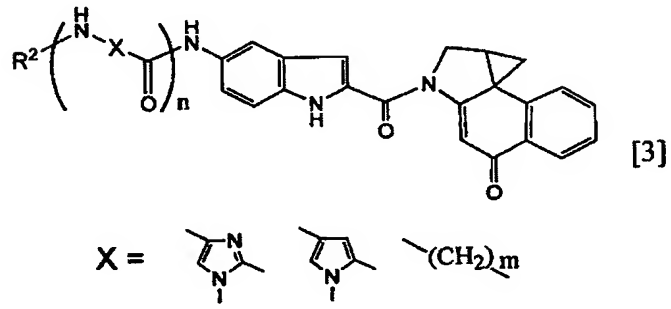
(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^1 represents an alkylating moiety; R^2 represents a pyrrole-imidazole polyamide compound

consisting from a hydrogen atom, a lower alkyl group or an acetyl group.)

[Claim 3]

The hairpin polyamide according to claim 1, wherein the hairpin polyamide represented by the general formula [1] is represented by general formula [3]:

[Chemical formula 3]

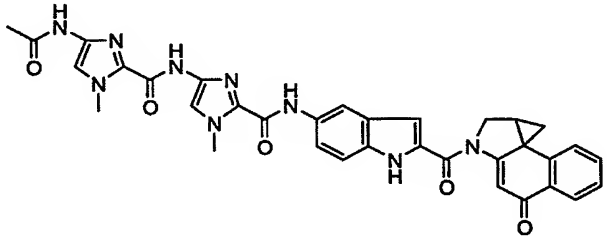


(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^1 represents an alkylating moiety; R^2 represents a pyrrole-imidazole polyamide compound consisting from a hydrogen atom, a lower alkyl group or an acetyl group.)

[Claim 4]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

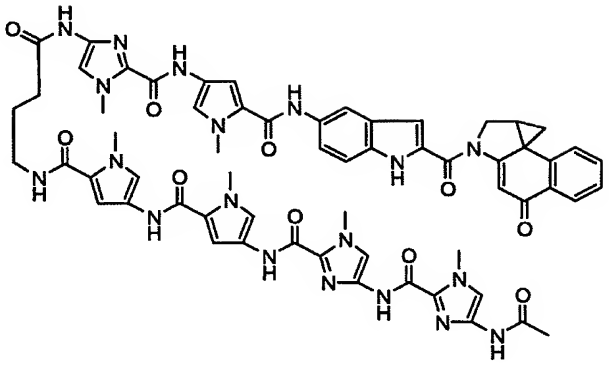
[Chemical formula 4]



[Claim 5]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

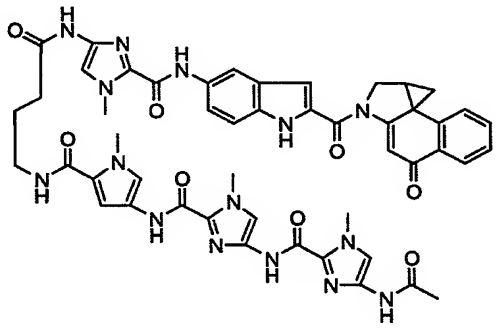
[Chemical formula 5]



[Claim 6]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

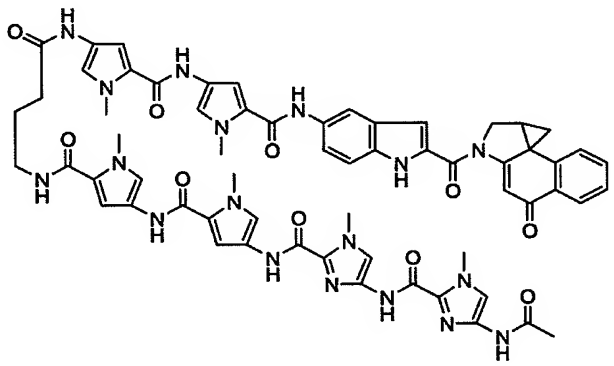
[Chemical formula 6]



[Claim 7]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

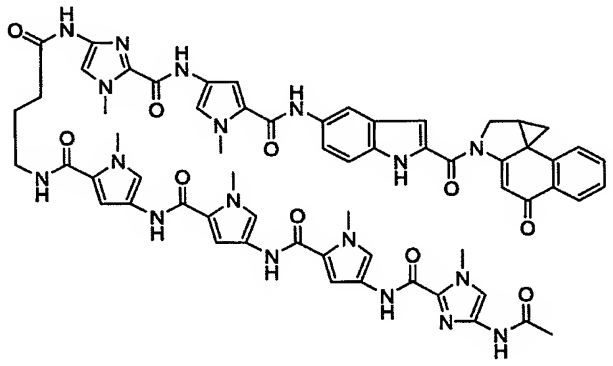
[Chemical formula 7]



[Claim 8]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

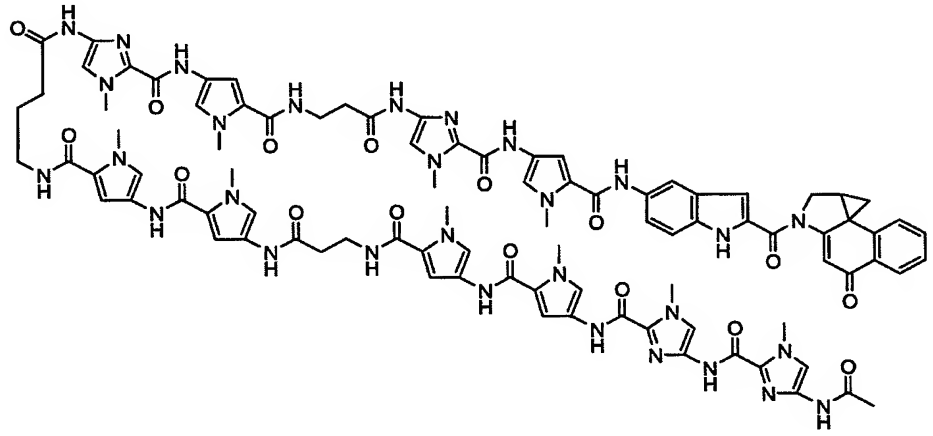
[Chemical formula 8]



[Claim 9]

The hairpin polyamide according to claim 3, wherein the hairpin polyamide represented by general formula [3] is represented by the following formula:

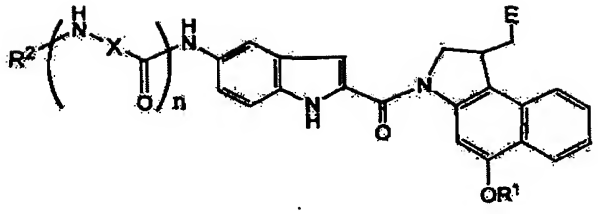
[Chemical formula 9]



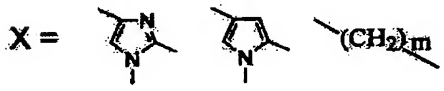
[Claim 10]

The hairpin polyamide according to claim 1, wherein the hairpin polyamide represented by general formula [1] is represented by general formula [4]:

[Chemical formula 10]



[4]

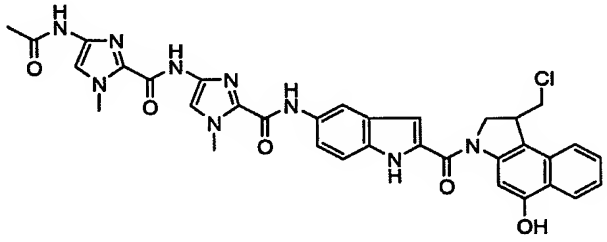


(wherein R^1 represents a hydrogen atom or a water-soluble estel derivatives of a peptide chain, a carbohydrate chain, a polyethylene glycol group, or the like; R2 represents a hydrogen atom, an alkyl group or an acetyl group; m and n are each an integer of 0 to 10; E represents an easily detachable substituent, such as a halogen atom, a mesyl group, or a tosyl group; X represents a pyrrole-imidazole polyamide compound optionally and independently selected from the structures described above.)

[Claim 11]

The hairpin polyamide according to claim 10, wherein the hairpin polyamide represented by general formula [4] is represented by the following formula:

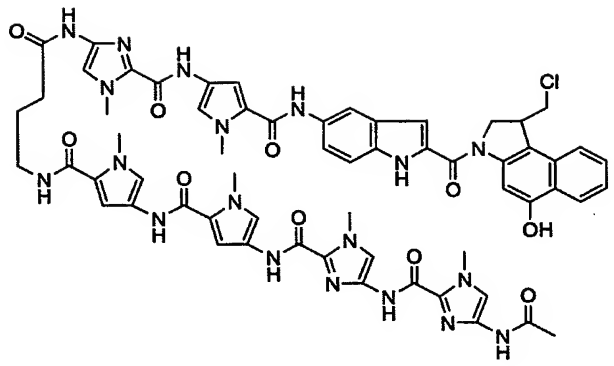
[Chemical formula 11]



[Claim 12]

The hairpin polyamide according to claim 10, wherein the hairpin polyamide represented by general formula [4] is represented by the following formula:

[Chemical formula 12]



[Claim 13]

A drug containing a hairpin polyamide according to claims 1 to 12, wherein the drug suppresses the expression of a gene.

[Claim 14]

A drug containing a hairpin polyamide according to claims 1 to 12, wherein the drug activates the expression of a gene.

[Claim 15]

The drug according to claims 13 and 14, wherein the gene is an abnormal gene.

[Claim 16]

The drug according to claims 13 and 14, wherein the gene is a single nucleotide polymorphism (SNPs).

[Claim 17]

A drug containing a hairpin polyamide according to claims 1 to 12, wherein the drug has the anticancer activity.

[Document] Specification

[Title of the Invention] NOVEL INDOLE DERIVATIVE FOR ALKYLATING SPECIFIC BASE SEQUENCE OF DNA AND ALKYLATING AGENT AND DRUG CONTAINING THE DERIVATIVE

[Field of the Invention]

[0001]

The present invention relates to a novel hairpin pyrrole-imidazole polyamide that particularly suppresses the occurrence of genetic abnormalities and has high anticancer activity and significantly high reactivity.

[Background Art]

[0002]

Now that the elucidation of the gene arrangement of humans has been substantially completed, many researchers have focused on a molecule having the ability to specifically bind to a particular base sequence. Dervan et al. found that an antiparallel oriented pyrrole (Py)–imidazole (Im) polyamide base-sequence-specifically binds to the minor groove of DNA (for example, see Non-Patent Document 1).

[Non-Patent Document 1] J. Am. Chem. Soc. 1997, 119, 7636

These molecules each have an association constant and specificity comparable to a transcription factor and the like. However, the regulation of gene expression is performed by inhibiting binding of the transcription factor. Thus, base sequences that can be targeted is significantly limited (for example, see Non-Patent Document 2).

[Non-Patent Document 2] J. Am. Chem. Soc. 2000, 122, 4856

The inventors have developed a hybrid molecule of a Py-Im polyamide bound to a segment A (Du) serving as an alkylating moiety of duocarmycin A,

which is an antibiotic, and filed an application (for example, Patent Document 1). The hybrid molecule selectively alkylated one site of a DNA fragment of 450 base pairs on the basis of the sequence recognition ability of the Py-Im polyamide (for example, see Non-Patent Document 3). However, completion of the reaction requires several days. Furthermore, the reaction efficiency was as low as several percent.

[Patent Document 1] International Publication No. WO00/15641

[Non-Patent Document 3] J. Am. Chem. Soc. 1999, 121, 4961

In contrast, the inventors found that ImPyLDu86 containing a vinyl linker (L) disposed between alkylating moiety and the Py-Im polyamide dimerizes and selectively reacts with both chains at sites thereof located 5 base pairs apart in a 5'-PyG(A/T)CPu-3' sequence (wherein Py represents a pyrimidine base; and Pu represents a purine base; the same shall apply hereinafter) at a low concentration to cause alkylation with an efficiency as high as 70%. That is, the inventors found considerable improvement in reactivity and efficiency by introducing the linker moiety (for example, see Non-Patent Document 4).

[Non-Patent Document 4] J. Am. Chem. Soc. 2000, 122, 1602

[Disclosure of Invention]

[Problems to be Solved by the Invention]

[0003]

However, the hybrid molecule described above has problems related to difficulty of synthesis, the lability of the linker moiety, the reactivity of the alkylating moiety, and the like.

Accordingly, it is an object of the present invention to provide a pyrrole-imidazole polyamide compound capable of being synthesized through fewer reaction steps than known hybrid molecules and having a combination of a high

reactivity in DNA alkylation and the ability to recognize a sequence.

[Means for Solving the Problems]

[0004]

The present invention relates to a hairpin polyamide compound having an alkylating moiety at a terminus of pyrrole-imidazole polyamide via an indole linker.

[0005]

The present invention relates to a drug that contains the hairpin polyamide described above and suppresses or activates the expression of a gene.

[0006]

The present invention relates to a drug that contains the hairpin polyamide described above and has anticancer activity.

[Effect of the invention]

[0007]

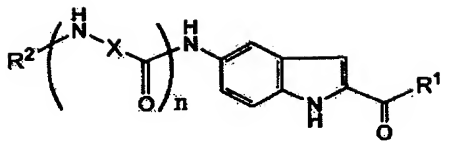
The present invention is a functional molecule selectively alkylating a specific base sequence present in a gene. The indole derivative can change a target base sequence by changing the configuration of imidazole, pyrrole, and the like in its molecule. Furthermore, a method for synthesizing the indole derivative is practical and highly applicable in view of the production of a wide variety of derivatives. Therefore, it is possible to logically design a drug on a genetic level, the drug being applicable to an important gene arrangement or an abnormal gene derived from a disease, such as cancer, in the human genome.

[Best Mode for Carrying Out the Invention]

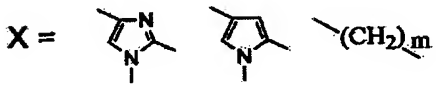
[0008]

Examples of the hairpin polyamide of the present invention include compounds represented by general formulae [1]:

[Chemical formula 13]



[1]

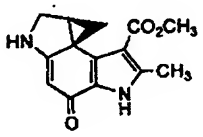


(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^1 represents an alkylating moiety; R^2 represents a pyrrole-imidazole polyamide compound consisting from hydrogen atom, alkyl group or acetyl group.)

[0009]

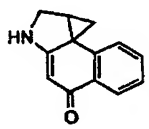
As the alkylating moiety represented by R^1 in general formula [1], any moiety having the abilities to recognize and alkylate a specific base sequence in DNA may be used. Examples of the moiety represented by formula [1] include a moiety of the segment of DU-86 (Du86)), which is CPI, represented by the following formula,

[Chemical formula 14]



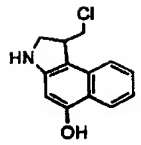
a moiety of 1,2,9,9a-tetrahydrocyclopropa[c]benzo[e]indol-4-one, which is CBI, represented by the following formula,

[Chemical formula 15]



and, a moiety of 1-chloromethyl-5-hydroxy-1,2-dihydro-3H-benzo[e]indole, which is a seco-CBI, represented by the following formula.

[Chemical formula 16]



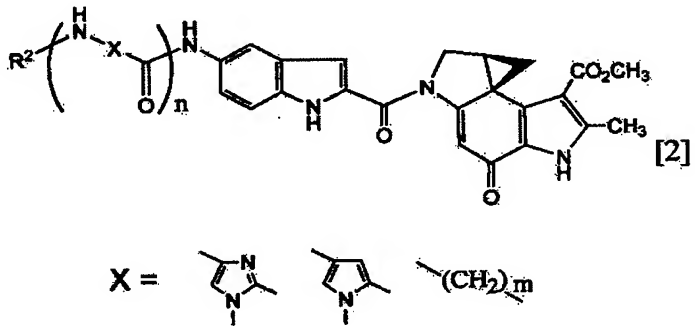
[0010]

The alkyl group represented by R^2 in general formula [1] has a carbon atom number of 1 to 20 and preferably 1 to 10. A linear or branched lower alkyl group having 1 to 6 carbon atoms is more preferred. Examples thereof include a methyl group, an ethyl group, an n-propyl group, an isopropyl group, an n-butyl group, an isobutyl group, a tert-butyl group, a pentyl group, and a hexyl group. Additionally, integer of m is usually 1 to 6 and preferably 2 to 3. Integer of n is usually 1 to 20 and preferably 2 to 7.

[0011]

Examples of the compounds represented by general formula [1] include a compound represented by the following general formula [2]:

[Chemical formula 17]



[2]

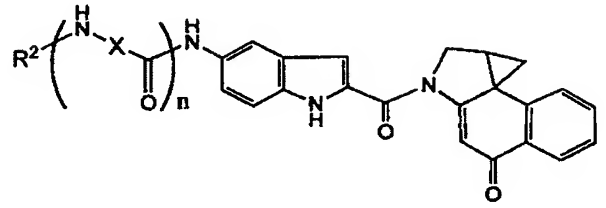
(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^2 represents a pyrrole-imidazole polyamide compound consisting from a hydrogen atom, a lower alkyl group or an acetyl group.)

[0012]

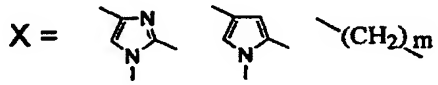
Examples of the compounds represented by general formula [1] include a

compound represented by the following general formula [3]:

[Chemical formula 18]



[3]

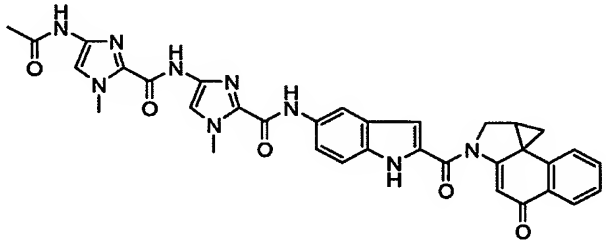


(wherein m and n are each an integer of 0 to 10; X is optionally and independently selected from the structures described above; R^2 represents a pyrrole-imidazole polyamide compound consisting from a hydrogen atom, a lower alkyl group or an acetyl group.)

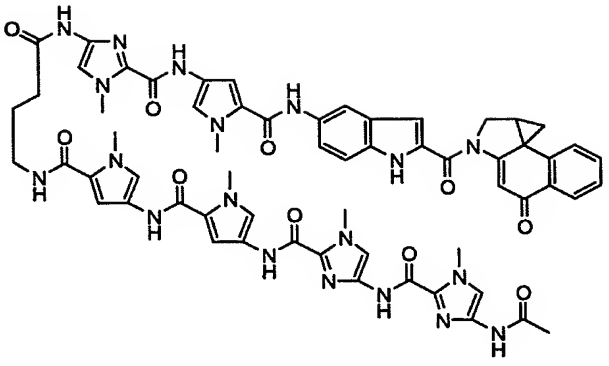
[0013]

Examples of the compounds represented by general formula [3] include hairpin polyamides or the like, represented by the following formulas:

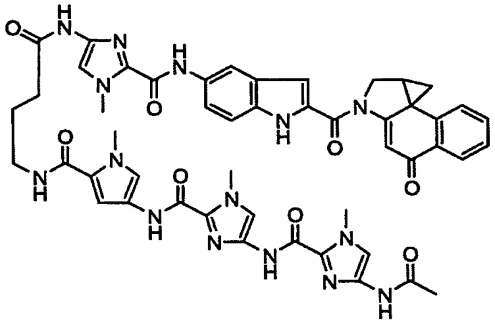
[Chemical formula 19]



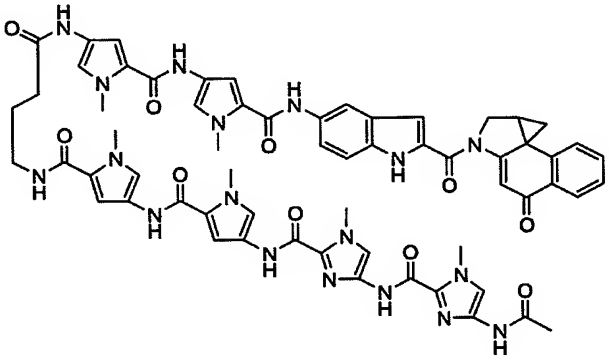
[Chemical formula 20]



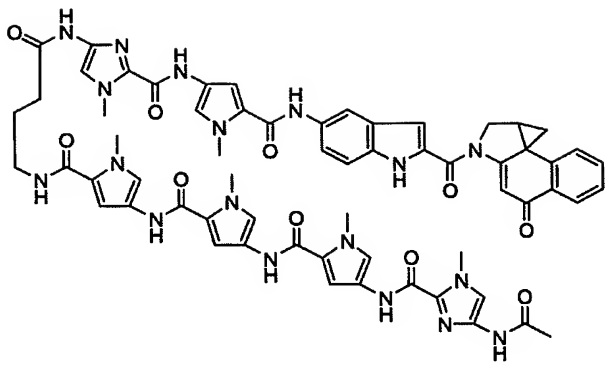
[Chemical formula 21]



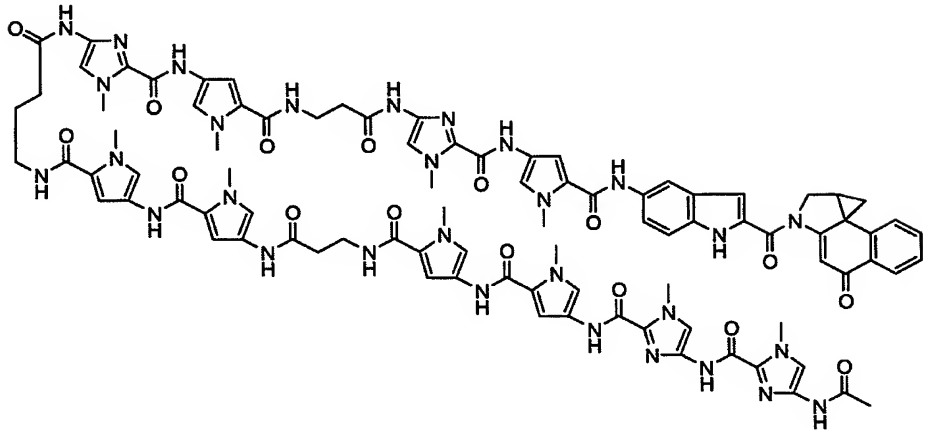
[Chemical formula 22]



[Chemical formula 23]



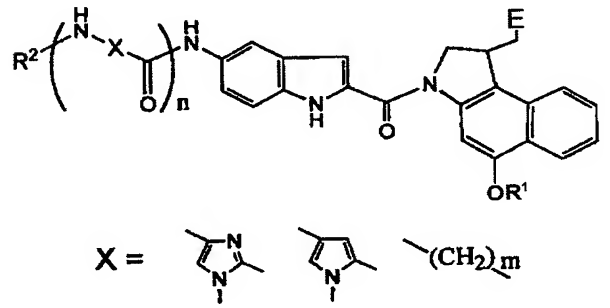
[Chemical formula 24]



[0014]

Examples of the compounds represented by general formula [1] include a hairpin polyamide represented by the following general formula [4]:

[Chemical formula 25]



[4]

(where R^1 represents a hydrogen atom or water-soluble estel derivatives; R^2 represents a hydrogen atom, an alkyl group or an acetyl group; m and n are

each an integer of 0-10; E represents an easily detachable substituent, such as a halogen atom, a mesyl group, or a tosyl group. X represents a pyrrole-imidazole polyamide compound optionally and independently selected from the structures described above.)

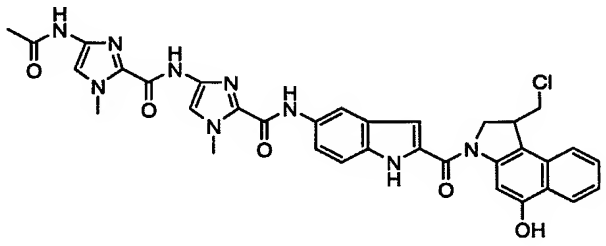
[0015]

Examples of halogen represented by E in general formula [4] include bromine, fluorine, and iodine. Preferably, halogen is chlorine. Examples of R^1 include peptide chains, proteins, monosaccharides, disaccharides, polysaccharides, and polyethylene glycols. Additionally, integer represented by m is usually 1 to 6 and preferably 2 to 3. Integer represented by n is usually 1 to 20 and preferably 2 to 7.

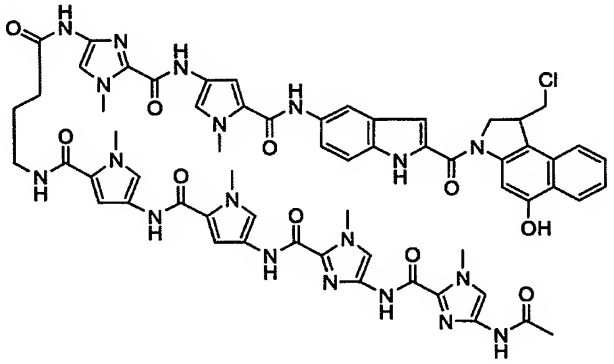
[0016]

Examples of the compounds represented by general formula [4] include hairpin polyamides or the like, represented by the following formulas:

[Chemical formula 26]



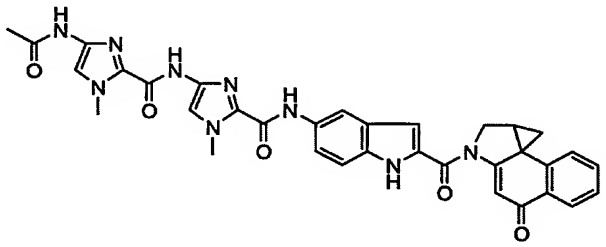
[Chemical formula 27]



[0017]

By taking compound represented by the following formula as an example, the outline of a method for synthesizing the indole derivative of the present invention will be described according to synthetic scheme 1.

[Chemical formula 28]

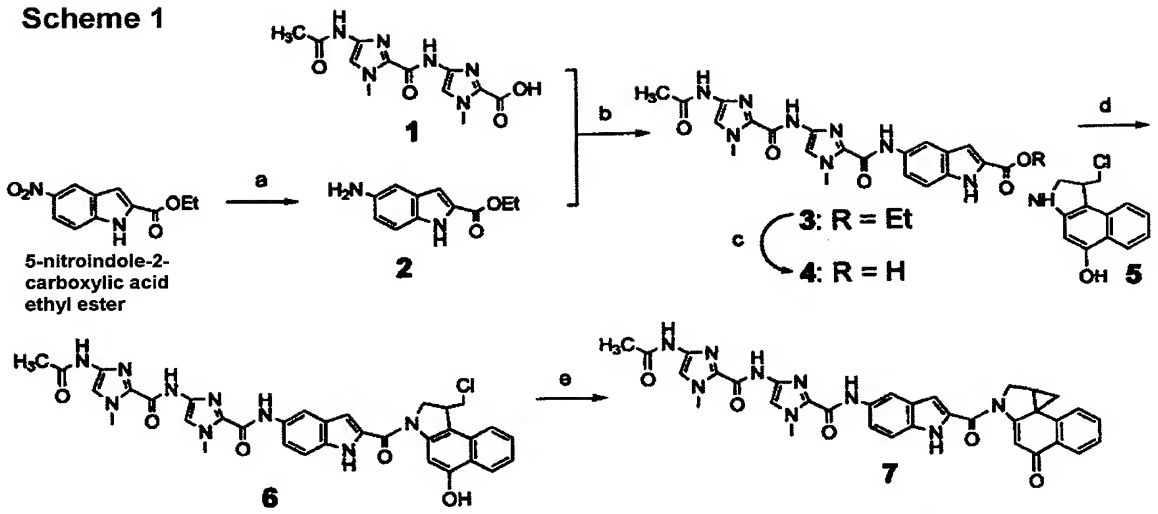


$AcImImCO_2H$ (1) and amine compound (2) prepared from 5-nitroindole-2-carboxylic acid ethyl ester by catalytic reduction are dissolved in dimethylformamide (DMF). O-(7-azabenzatriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) functioning as a condensation agent and diisopropylethylamine ($^iPr_2NEt$) are added to the resulting solution to perform a condensation reaction into Py-Im indole ethyl ester (3). Compound (3) is subjected to alkaline hydrolysis to prepare carboxylic acid (4). Next, carboxylic acid (4) thus prepared is coupled to seco-CBI (5) that can be synthesized from commercial-products, thereby preparing open-circular compound (6) as a compound of the present invention. Furthermore, open-circular compound (6) is treated with a weak alkali ($NaHCO_3$) in water to prepare circular compound (7) as a compound of the present invention. Compound (6) and (7) were separated and purified by reverse phase HPLC with linear gradient of 0.1% acetic acid and acetonitrile, and these structures were confirmed by ^1H-NMR and electrospray-mass spectrometry.

[0018]

[Chemical formula 29]

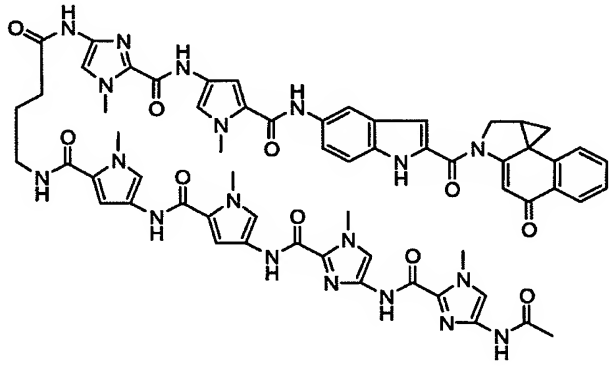
Scheme 1



Reaction conditions : (a) H_2, Pd-C, MeOH; (b) HATU, $^iPr_2NEt$, DMF; (c) aq. NaOH, MeOH ; (d) 5, EDCI, $NaHCO_3$, DMF; (e) 5% aq. $NaHCO_3$, H_2O.

[0019]

[Chemical formula 30]



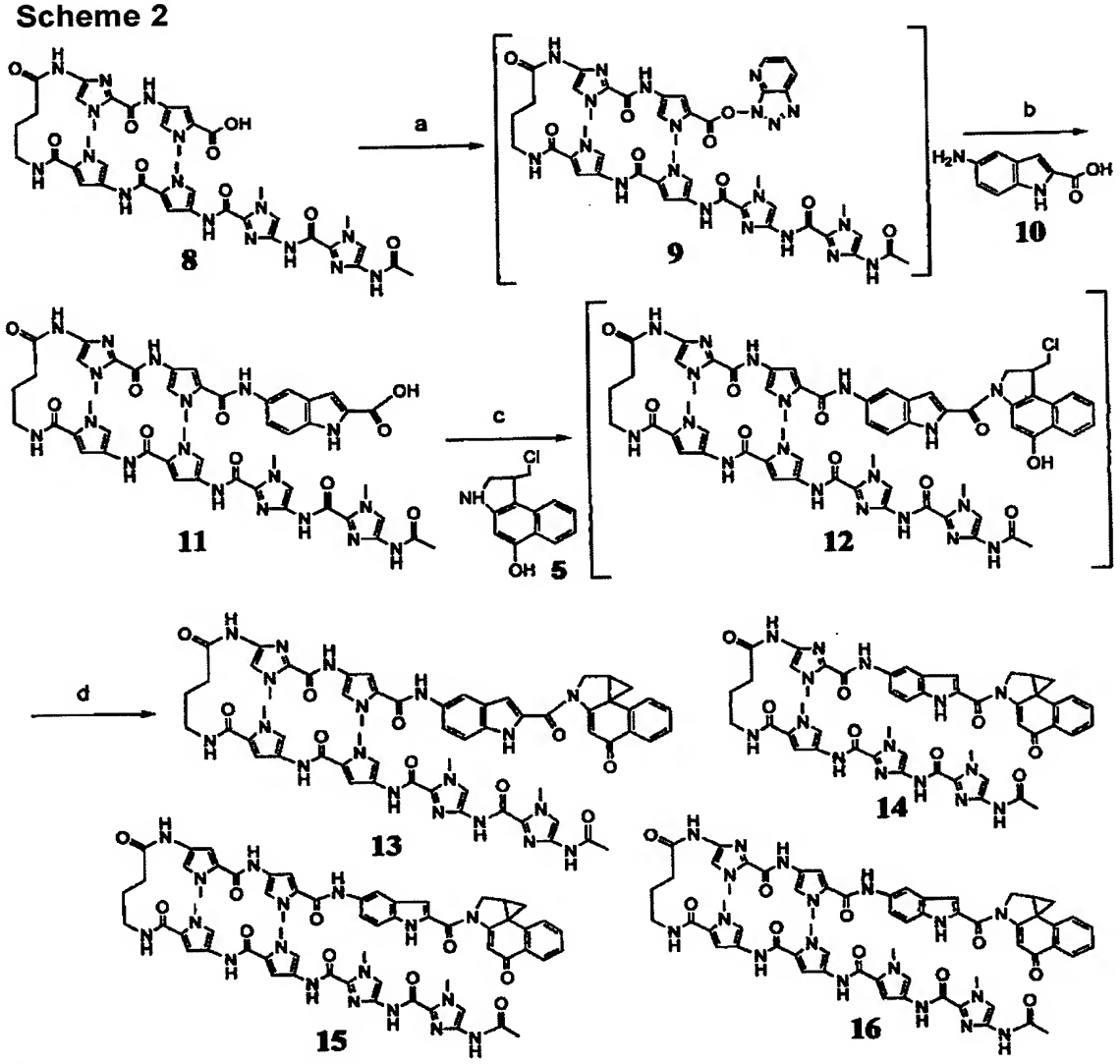
By taking compound described above as an example, the outline of a method for synthesizing the indole derivative of the present invention will be described according to synthetic scheme 2. By applying Fmoc solid-phase synthesis to a Py-Im polyamide using an oxime resin, carboxylic acid (8) corresponding to the above-described hairpin Py-Im polyamide is prepared. After carboxylic acid (8) is converted into active At ester (9) using HATU in the reaction system, aminoindolecarboxylic acid (10) is added thereto to synthesize indolecarboxylic acid (11) at a time. Py-Im polyamide is coupled to seco-CBI (5) that can be

prepared from commercially available 1,3-naphthalenediol as alkylation moiety, thereby preparing open-circular compound (12). Furthermore, open-circular compound (12) is treated with a weak alkali ($NaHCO_3$) in water to prepare circular compound (13) as the indole derivative of the present invention. Circular compounds (14), (15), (16), and the like as indole derivatives of the present invention can be prepared in the same way. Compounds were separated and purified by reverse phase HPLC with linear gradient of 0.1% acetic acid and acetonitrile, and these structures were confirmed by ^1H-NMR and electrospray-mass spectrometry.

[0020]

[Chemical formula 31]

Scheme 2



Reaction conditions

: (a) HATU, $^{i}Pr_2NEt$, DMF ; (b) **10**, $^{i}Pr_2NEt$, DMF ; (c) **5**, EDCI, $NaHCO_3$, DMF ; (d) 5% aq. $NaHCO_3$

[0021]

An active component compound used in the present invention may be a pharmaceutically acceptable salt thereof. Examples of the salt include commonly used salts, such as salts with acids, e.g., hydrochlorides, phosphates, citrates, and sulfonates, and salts with organic bases, e.g., methylamine and ethylenediamine.

[0022]

The compound of the present invention or the pharmaceutically

acceptable salt thereof has advantages described as follows:

(a) the effect of the compound can be expected in a small amount because of high uptake by cancer cells;

(b) the compound can be expected to be orally absorbed because the compound is stable in an acid or an alkali;

(c) it is possible to control the expression of a specific gene; and

(d) no damage to normal cells is caused compared with known DNA alkylating agents.

[0023]

The compound of the present invention is expected to have a therapeutic effect on various diseases by controlling the expression of a gene. In particular, the drug is useful for the treatment and prevention of cancer and can be used for deep cancer.

[0024]

When the compound of the present invention is used as an anticancer agent, the drug is formed into a commonly used formulation, such as an injection, a tablet, a powder, or a capsule. In making the formulation, a commonly used, pharmaceutically acceptable carrier, such as a binder, a lubricant, a disintegrator, a solvent, a dispersant, a stabilizer, a pigment, or a flavor, may be used. The content of the compound in the formulation is different depending on types of compound and formulation and is usually 0.2 to 50 percent by weight.

[0025]

The dosage of the inventive compound as an anticancer agent is different depending on age, weight, pathology, a therapeutic effect, an administration method, administration timing, dosing days, and the duration of drug administration. Usually, 10 to 400 mg of the drug is administered once a day for 1, 2, or 3 weeks and 1 week following drug withdrawal as one course of

treatment. The administration is repeated.

[0026]

The compound is used for cancer that causes further expression of abnormal genes, regardless of type of cancer. In particular, the drug is used for abnormality of the genes in cancer on which a known anticancer agent does not easily exert an effect.

[EXAMPLES]

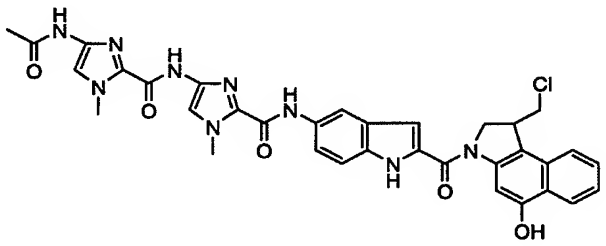
[0027]

While the present invention will be specifically described by examples, the present invention is not limited to these examples.

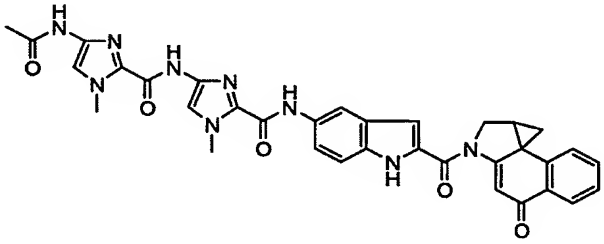
[Example 1]

Ability to Alkylate Linear DNA (400 bp or more)

[Chemical formula 32]



[Chemical formula 33]

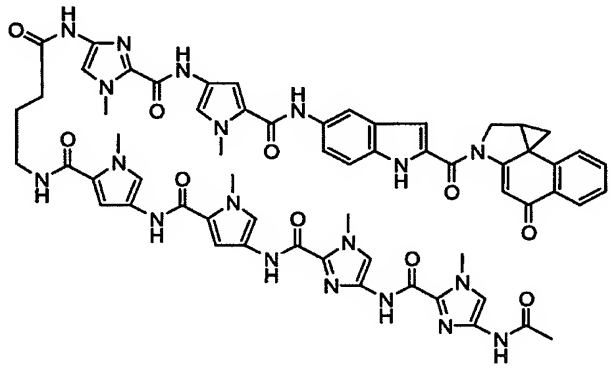


The reaction of above-described compounds (6) and (7) with DNA was studied using the long-chain DNA (pUC-II'). An alkylation reaction was performed at 23°C for 8 hours. The sequence alkylated was analyzed by polyacrylic gel

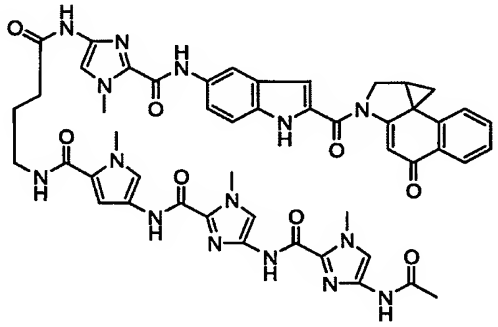
electrophoresis for sequence determination. Fig. 1 shows the results. Open-circular compound (6) and circular compound (7) had the same sequence specificity and was selectively alkylated adenine located at site 1 (5'-CGGCCA-3') at nanomolar concentrations. This sequence specificity can be explained by a model in which each compound dimerizes in the DNA minor groove to recognize the base sequence.

[0028]

[Chemical formula 34]



[Chemical formula 35]

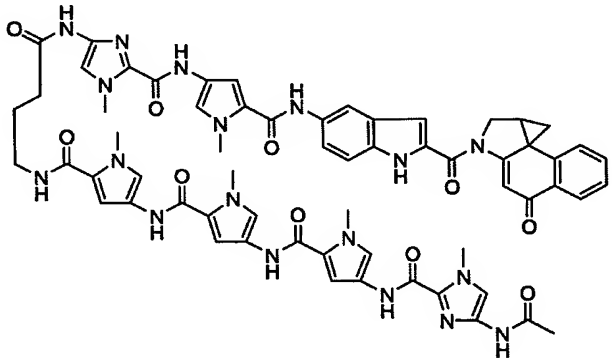


The reaction of above-described compounds (13) and (14) with DNA was studied using the long-chain DNA (pUC-II and pUC-I'). An alkylation reaction was performed for 8 hours. The sequence alkylated was analyzed by polyacrylic gel electrophoresis for sequence determination. Fig. 2 shows the results. As a result, in both compounds (13) and (14), efficient DNA alkylation comparable with that of a known Py-Im polyamide having a vinyl linker was observed at

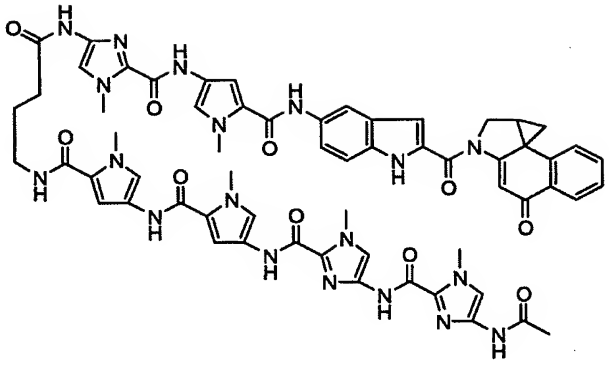
nanomolar concentrations. However, the difference in sequence recognition ability was observed. In compound (14), alkylation of site 4 (5'-AGCCA-3'), which was a match sequence, was observed. On the other hand, single-base-pair mismatch sequences in site 1 (5'-AGCTA-3'), site 2 (5'-AGTCA-3'), site 5 (5'-TACCA-3'), and the like were also observed. In contrast, in compound (13), alkylation in only site 3 (5'-TGACCA-3') and site 6 (5'-AGTCCA-3'), which were match sequences, was observed. From the stand point of the sequence recognition ability, the molecular design of compound (13) was excellent. Interestingly, although pUC-I' contained a site (5'-TGCCG-3') corresponding to a match sequence of compound (14), no alkylation of guanine was observed. In consideration of the results shown in Fig. 2, it is believed that the combination of the indole linker and CBI alkylates adenine at the N-3 position with highly sequence specificity. In sequence recognition, it is believed that the indole linker adjusts the distance between the cyclopropane ring of CBI and the N-3 position of adenine so as to cause alkylation while functioning as like Py (Fig. 3).

[0029]

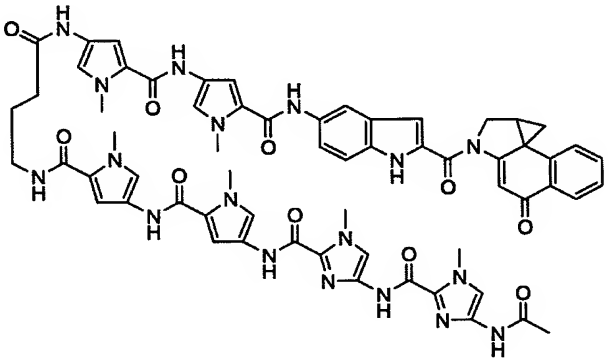
[Chemical formula 36]



[Chemical formula 37]



[Chemical formula 38]



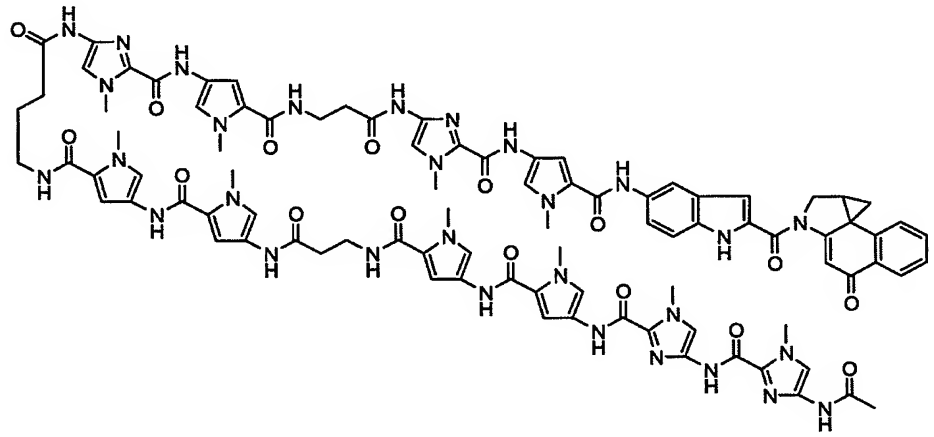
The reaction of compounds (13), (15), and (16) with DNA was studied using the long-chain DNA (λ-F10906). Compounds (15) and (16) having the ability to recognize sequence different from that of compound (13) were synthesized by utilizing solid phase synthesis according to the same way. Then, the DNA-alkylating ability of each compound was evaluated. Fig. 4 shows the results. Sequence-specific DNA alkylation (site 2 and 3: 5'-TGACCA-3', site 4: 5'-AGACCA-3', site 5: 5'-TGTCCA-3') by compound (13) was observed at nanomolar concentrations as in the results shown in Fig. 2. Furthermore, Sequence-specific DNA alkylation (site 6: 5'-AATCCA-3', site 7: 5'-TAACCA-3') by compound (16) was also observed at nanomolar concentrations. In compound (15), alkylation of an only mismatch sequence (site 1: 5'-TGCTCA-3') was observed. These results indicate the possibility that in the molecular

design of the hairpin Py-Im polyamide including the indole linker, alkylating agents that are capable of recognizing intended sequences can be produced by only changing the configuration of Py-Im.

[0030]

Establishment of the synthetic pathway using the indole linker made it possible to synthesize an alkylating agent, which cannot be synthesized using a known vinyl linker, having the ability to recognize a longer base sequence. For example, a Py-Im carboxylic acid having the ability to recognize a long base sequence was prepared by Fmoc solid-phase synthesis for a Py-Im polyamide using an oxime resin, thus making it possible to synthesize compound (17) described below.

[Chemical formula 39]



Alkylation of the DNA fragment derived from pQBI63 was studied. The results demonstrated that compound (17) efficiently alkylated 1,000-base-pair DNA at nanomolar concentrations (Fig. 5). Furthermore, alkylation (site 2) due to 1-bp-mismatch recognition was observed. However, alkylation of AT-rich base sequences (sites 1 and 3) was also observed. In the Py-Im polyamide, such as compound (17), having the ability to recognize a long base sequence, the maintenance of high specificity is an important issue. It is believed that further optimization of the current molecular design makes it possible to produce a practical alkylating agent having the ability to recognize a sequence

having 10 base pair or more with excellent versatility.

[Example 2]

[0031]

Stability of Hairpin Polyamide

The Py-Im polyamide containing the indole linker was evaluated for stability by product analysis on HPLC using compounds (6) and (7). Fig. 6 shows the results. It was found that compound (6) was not hydrolyzed at all in the acidic aqueous HCl solution (pH = 1) (left standing at 37°C for 24 hours). Compound (6) was converted into compound (7) (30 minutes: 50%, 2 hours: 78%) in the alkaline aqueous $NaHCO_3$ solution (pH = 9) (left standing at 37°C for 24 hours). No other hydrolysates were formed. Compound (7) was readily converted into compound (6) (30 minutes: 96%) in the acidic aqueous HCl solution (pH = 1) (37°C). That is, it was found that the Py-Im polyamide containing the indole linker was stably present in a seco-CBI form like compound (6) under the acidic condition (pH = 1) and in a CBI form like compound (7) under the alkaline condition (pH = 9).

[Example 3]

[0032]

Anticancer Activity in vitro by Hairpin Polyamide

HCT-116 (human colon cancer-derived cell), HeLa (human cervical cancer-derived cell), HLC-2 (human lung cancer-derived cell), and SH-SY-5Y (human neuroblast-derived cell) were treated with AcImImPyPy-γ-ImPy-Indole-CBI (13) (10^{-5} to 10^{-8} M with 0.1% DMF) for 48 hours to evaluate the effect of the compound as an anticancer agent. As a result, 50% cell growth inhibitory concentration (IC_{50}) against HCT-116, HeLa, HLC-2, and SH-SY-5Y were 7.42×10^{-8}, 5.97×10^{-8}, 5.35×10^{-8}, and 7.43×10^{-9} M, respectively. Similarly, evaluation of 293T (human kidney-derived normal cell) and WI-38 (human normal fibroblast) were made, and IC_{50} was 6.99×10^{-8} and 6.79×10^{-8} M,

respectively. The results indicated that the anticancer activity of the compound 13 against the cancerous cell lines was about 10 times that against the normal cell lines (IC_{50} values were calculated with reference to a system as a control treated with 0.1% DMF for 48 hours).

[Embodiments]

[Embodiment 1]

Synthesis of 5'-Texas Red-Labeled DNA Fragment of 450 bp

[0033]

A DNA fragment (pUC-I') was prepared by a PCR method using a 5'-Texas Red-labeled 20-base-pair primer, i.e., 5'-TexRed-AGAATCAGGGGATAACGCAG-3' (pUC18 forward, 780-799), and a 20-base-pair primer, i.e., 5'-TTACCAGTGGCTGCTGCCAG-3' (pUC18 reverse, 1459-1478), puc18 being used as a template.

A DNA fragment (pUC-II) was prepared by a PCR method using a 5'-Texas Red-labeled 21-base-pair primer, i.e., 5'-TexRed-TGCTGGCCTTTTGCTCACATG-3' (pUC18 reverse, 1861-1881), and a 18-base-pair primer, i.e., 5-TGTAAAACGACGGCCAGTG-3' (pUC18 forward, 378-395), puc18 being used as a template. In the same way, a DNA fragment (pUC-II') was prepared by a PCR method using the 5'-Texas Red-labeled 18-base-pair primer (pUC18 forward, 378-395) and the 21-base-pair primer (pUC18 reverse, 1861-1881), puc18 being used as a template.

A DNA fragment (λ-F10906) was prepared by a PCR method using a 5'-Texas Red-labeled 23-base-pair primer, i.e., 5'-TexRed-ATCAGGGCAACTCAACCCTGTCC-3' (λ-DNA forward, 10960-10982), and a 20-base-pair primer, i.e., 5'-CAGGACGACCAATATCCAGC-3' (λ-DNA reverse, 37007-37026), λ-DNA being used as a template.

A DNA fragment (pQBI63) was prepared by a PCR method using a 20-base-pair primer, i.e., 5'-GGTGATGTCGGCGATATAGG-3', and a 5'-Texas Red-

labeled 20-base-pair primer, i.e., 5'-TexRed-CCCCAAGGGGTTATGCTAGT-3', pQBI63 plasmid being used as a template. The resulting each DNA fragment was purified by filtration using Suprec-02. The concentration of each resulting product was determined by measuring UV absorption.

[Embodiment 2]

Analysis by polyacrylamide gel electrophoresis

[0034]

A standard reaction solution containing the 5'-Texas Red-end-labeled DNA fragment (10 nM), DMF (10% (v/v)), and an agent having a concentration described above in a sodium phosphate buffer (5 mM, total volume: 10 μL, pH = 7.0) was charged into a microfuge tube (Eppendorf) and left standing at 23°C for 8 hours.

After completion of the reaction, the reaction was quenched by the addition of calf thymus DNA (1 mM, 1 μL). The resulting mixture was shaken at 90°C for 5 minutes. The DNA obtained by centrifugation under reduced pressure was dissolved by the addition of 8 μL of loading dye (fushin red solution in DMF), shaken at 94°C for 20 minutes, and then immediately cooled to 0°C. A 2-μL aliquot was separated electrophoretically on a 6% denaturing polyacrylamide gel using a Hitachi 5500-S DNA sequencer.

[Embodiment 3]

Analysis of stability under acidic and alkaline conditions by HPLC

[0035]

A reaction solution containing compound (6) or (7) (100 μM) in a total volume of 10 μL of an aqueous solution of 5% HCl (pH = 1, DMF:H_2O = 1:9) or an aqueous solution of 5% $NaHCO_3$ (pH = 9, DMF:H_2O = 1:9) was charged into a microfuge tube (Eppendorf) and left standing at 37°C. After 30 minutes, 2 hours, and 24 hours, analysis of compound (6) (16.8 min) and compound (7) (15.1 min) was performed by HPLC (linear gradient of acetonitrile 0-100% in 50

mM ammonium formate, 20 min, flow rate: 1.0 mL/min, 254 nm).

[Embodiment 4]

Evaluation of anticancer activity

[0036]

HCT-116, HeLa, HLC-2, and SH-SY-5Y cell lines were seeded into a 96-well flat bottom plate in complete media [RPMI1640 (HCT-116, SH-SY-5Y; Sigma-Aldrich Co.) each containing 10% fetal bovine serum and Dulbecco's modified minimal media (HeLa, HLC-2, 293T, WI-38; Sigma-Aldrich Co.)] at cell densities of 4.0×10^3, 3.6×10^3, 1.6×10^3, 7.0×10^2, 5.0×10^2, and 8.0×10^2 cells/well and were precultured for 24 hours in a CO_2 incubator. The media were replaced with complete media containing 0.1% DMF and 10^{-5} to 10^{-8} M AcImImPyPy-γ-ImPy-Indole-CBI (13). Incubation was performed for 48 hours in the CO_2 incubator. Then, 10 μL/well of a Cell Counting Kit-8 (Dojindo Laboratories) was added. The specimens were left standing for 2 hours in the CO_2 incubator. Absorbance was measured with a microplate reader MPR-A4I (Tosoh Corp.).

[Embodiment 5]

Synthesis of Compounds (6) and (7) (Scheme 1)

[0037]

(1) Synthesis of AcImImCO_2H (1)

Compound (1) was synthesized according to a method described in literature [(a) Tao, Z. -F.; Fujiwara, T.; Saito, I.; Sugiyama, H. J. Am. Chem. Soc. 1999, 121, 4961. (b) Tao, Z. -F.; Saito, I.; Sugiyama, H. J. Am. Chem. Soc. 2000, 122, 1602. (c) Bando, T.; Narita, A.; Saito, I.; Sugiyama, H. Chem. Eur. J. 2002, 8, 4781].

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 10.37 (s, 1H; NH), 9.60 (s, 1H; NH), 7.63 (s, 1H; CH), 7.48 (s, 1H; CH), 3.96 (s, 3H; NCH_3), 3.93 (s, 3H; NCH_3), 2.03 (s, 3H; $COCH_3$);

ESIMS m/e calcd. for $C_{12}H_{15}N_6O_4$

$[M^+ + H]$ 307.1, found 307.3

[0038]

(2) Synthesis of H_2N-Indole-CO_2Et (2)

Compound (2) was synthesized from commercially available 5-nitroindole-2-carboxylic acid ethyl ester as a starting material by catalytic reduction with Pd-C in a hydrogen atmosphere. Compound (2) was used as a starting material for synthesizing compound (3) without purification.

[0039]

(3) Synthesis of AcImIm-Indole-CO_2Et (3)

Compound (1) (305 mg, 1.05 mmol) and compound (2) (215 mg, 1.05 mmol) were dissolved in DMF (2 mL). Then, $^iPr_2NEt$ (550 μL, 3.15 mmol) and HATU (480 mg, 1.26 mmol) were added to the resulting solution. The resulting mixture was stirred at room temperature for 15 hours in a nitrogen atmosphere. After completion of the reaction, the solvent in the reaction solution was distilled off under reduced pressure. The resulting residue was purified by silica-gel column chromatography (3% to 5% MeOH in CH_2Cl_2, gradient elution), followed by removing the solvent to yield a yellow powder (3) (326 mg, 52%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.83 (s, 1H; NH), 10.32 (s, 1H; NH), 10.08 (s, 1H; NH), 9.40 (s, 1H; NH), 8.16 (s, 1H; CH), 7.60 (s, 1H; CH), 7.56 (d, 1H, J = 9.0 Hz; CH), 7.50 (s, 1H; CH), 7.40 (d, 1H, J = 9.0 Hz; CH), 7.12 (s, 1H; CH), 4.33 (q, 2H, J = 7.0 Hz; OCH_2), 4.01 (s, 3H; NCH_3), 3.98 (s, 3H; NCH_3), 2.03 (s, 3H; $COCH_3$), 1.33 (t, 3H, J = 7.0 Hz; CH_3);

ESIMS m/e calcd. for $C_{23}H_{25}N_8O_5$

$[M^+ + H]$ 493.2, found 493.2

[0040]

(4) Synthesis of AcImIm-Indole-CO_2H (4)

MeOH (8 mL) and 1 N aqueous sodium hydroxide solution (8 mL) were

added to compound (3) (326 mg, 0.66 mmol). The resulting mixture was stirred at room temperature for 30 minutes. After removal of MeOH under reduced pressure, 10% aqueous HCl solution was added thereto at 0°C to make the mixture acidic (pH = 2). The resulting precipitate was collected by filtration with a KIRIYAMA funnel, washed with water, and dried to yield compound (4) (297 mg, 96%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.71 (s, 1H; NH), 10.35 (s, 1H; NH), 10.08 (s, 1H; NH), 9.49 (s, 1H; NH), 8.13 (d, 1H, J = 1.0 Hz; CH), 7.60 (s, 1H; CH), 7.54 (dd, 1H, J = 2.0 and 9.0 Hz; CH), 7.49 (s, 1H; CH), 7.38 (d, 1H, J = 9.0 Hz; CH), 7.06 (d, 1H, J = 2.0 Hz; CH), 4.01 (s, 3H; NCH_3), 3.97 (s, 3H; NCH_3), 2.03 (s, 3H; $COCH_3$);

ESIMS m/e calcd. for $C_{21}H_{21}N_8O_5$

$[M^+ + H]$ 465.2, found 465.2

[0041]

(5) Synthesis of seco-CBI (5)

Compound (5) was synthesized as a hydrochloride according to a method described in literature [(a) Boger, D. L.; Yun, W. Y.; Teegarden, B. R. J. Org. Chem. 1992, 57, 2873. (b) Boger, D. L.; McKie, J. A. J. Org. Chem. 1995, 60, 1271. (c) Boger, D. L.; Ishizaki, T.; Kitos, P. A.; Suntornwat, O. J. Org. Chem. 1990, 55, 5823]. Spectrum data were reported in the literature.

[0042]

(6) Synthesis of AcImIm-Indole-seco-CBI (6)

seco-CBI (5) (32.7 mg, 0.13 mmol), EDCI (50.3 mg, 0.26 mmol), and $NaHCO_3$ (41.9 mg, 0.52 mmol) were charged into a reaction vessel containing compound (4) (60.3 mg, 0.13 mmol). The resulting mixture was dissolved in DMF (700 μL) and stirred at room temperature for 8 hours in a nitrogen atmosphere. After confirmation of completion of the reaction, the solvent was distilled off. The resulting residue was purified by silica-gel column

chromatography (5% to 10% MeOH in CH_2Cl_2, gradient elution). The resulting solid was washed with $CHCl_3$ and dried to yield a brown powder (6) (85.2 mg, 96%).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.71 (s, 1H; NH), 10.43 (s, 1H; OH), 10.34 (s, 1H; NH), 10.11 (s, 1H; NH), 9.41 (s, 1H; NH), 8.16 (s, 1H; CH), 8.12 (d, 1H, J = 8.0 Hz; CH), 7.97 (brs, 1H; CH), 7.85 (d, 1H, J = 8.0 Hz; CH), 7.62 (m, 1H; CH), 7.61 (s, 1H; Im-H), 7.52 (t, 1H, J = 8.0 Hz; CH), 7.51 (s, 1H; Im-H), 7.45 (d, 1H, J = 9.0 Hz; CH), 7.36 (t, 1H, J = 8.0 Hz; CH), 7.19 (s, 1H; CH), 4.81 (t, 1H, J = 11.0 Hz; NCHH), 4.56 (d, 1H, J = 11.0 Hz; NCHH), 4.23 (brt, 1H; CH), 4.03 (s, 3H; NCH_3), 3.99 (s, 3H; NCH_3), 3.87 (dd, 2H, J = 7.0 and 11.0 Hz; CH_2), 2.04 (s, 3H; $COCH_3$);

ESI-TOFMS m/e calcd. for $C_{34}H_{31}ClN_9O_5$

$[M^+ + H]$ 680.22, found 680.23

[0043]

(7) Synthesis of AcImIm-Indole-CBI (7)

First, 5% aqueous $NaHCO_3$ (100 μL) was added to a solution of compound (6) (6.3 mg, 9.28 mmol) in DMF (150 μL). The resulting mixture was stirred at room temperature for 1 hour in a nitrogen atmosphere. After confirmation of completion of the reaction, the solvent was distilled off. The resulting residue was purified by silica-gel column chromatography (5% to 15% MeOH in CH_2Cl_2, gradient elution). The solvent was distilled off. The resulting residue was dried to yield a brown powder (7) (6.0 mg, quant.).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.85 (s, 1H; NH), 10.35 (s, 1H; NH), 10.16 (s, 1H; NH), 9.41 (s, 1H; NH), 8.17 (s, 1H; CH), 8.00 (d, 1H, J = 8.0 Hz; CH), 7.94 (s, 1H; CH), 7.62 (s, 1H; Im-H), 7.52 (m, 2H; CHx2), 7.51 (s, 1H; Im-H), 7.43 (m, 2H; CH), 7.25 (d, 1H, J = 8.0 Hz; CH), 6.95 (s, 1H; CH), 4.64 (m, 1H; NCHH), 4.49 (d, 1H, J = 10.0 Hz; NCHH), 4.02 (s, 3H; NCH_3), 3.98 (s, 3H; NCH_3), 3.27 (brt, 1H; CH), 2.03 (s, 3H; $COCH_3$), 1.75 (m, 1H; CHH), 1.70 (m, 1H; CHH);

ESI-TOFMS m/e calcd. for $C_{34}H_{30}N_9O_5$

$[M^+ + H]$ 644.23, found 644.21

[Embodiment 6]

Synthetic Reaction of Compound (13), (14), (15), and (16) (Scheme 2)

[0044]

(1) Synthesis of AcImImPyPy-γ-ImPyCO$_2$H (8)

Compound (8) was prepared by an Fmoc solid-phase synthesizer. Purification was performed by HPLC with a Chemcobond 5-ODS-H column (conditions: linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting compound was used as a starting material for synthesis.

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 10.32 (s, 1H; NH), 10.29 (s, 1H; NH), 10.23 (s, 1H; NH), 10.00 (s, 1H; NH), 9.91 (s, 1H; NH), 9.32 (s, 1H; NH), 8.02 (s, 1H; NH), 7.56 (s, 1H; Im-H), 7.50 (s, 1H; Im-H), 7.45 (s, 1H; Py-H), 7.44 (s, 1H; Im-H), 7.27 (s, 1H; Py-H), 7.16 (s, 1H; Py-H), 7.14 (s, 1H; Py-H), 6.92 (s, 1H; Py-H), 6.89 (s, 1H; Py-H), 4.00 (s, 3H; NCH_3), 3.97 (s, 3H; NCH_3), 3.93 (s, 3H; NCH_3), 3.84 (s, 3H; NCH_3), 3.81 (s, 3H; NCH_3), 3.79 (s, 3H; NCH_3), 3.19 (m, 2H; CH_2), 2.34 (m, 2H; CH_2), 2.03 (s, 3H; $COCH_3$), 1.78 (m, 2H; CH_2);

ESI-TOFMS m/e calcd. for $C_{39}H_{45}N_{16}O_9$

$[M^+ + H]$ 881.35, found 881.36

[0045]

(2) Synthesis of H_2N-Indole-CO_2H (10)

Compound (10) was synthesized from commercially available 5-nitroindole-2-carboxylic acid ethyl ester as a starting material by a two-step reaction ((i) alkali hydrolysis with 1 N NaOH; and (ii) catalytic reduction with Pd-C in a hydrogen atmosphere).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.22 (s, 1H; NH), 7.11 (d, 1H, J = 8.5 Hz; CH), 6.75 (d, 1H, J = 2.0 Hz; CH), 6.67 (s, 1H; CH), 6.65 (dd, 1H, J = 2.0 and 8.5 Hz; CH), 3.31 (brs, 2H; NH_2, H_2O)

[0046]

(3) Synthesis of AcImImPyPy-γ-ImPy-Indole-CO_2H (11)

Compound (8) (3.5 mg, 3.98 mmol) prepared by a solid-phase synthesis was dissolved in DMF (75 μL). Then, iPr_2NEt (1.4 μL, 8.04 mmol) and HATU (1.4 mg, 3.68 mmol) were added to the resulting solution. The resulting mixture was stirred at room temperature for 4 hours in a nitrogen atmosphere. After confirmation of completion of the reaction, compound (10) (1.3 mg, 7.38 mmol) and $^{i}Pr_2NEt$ (1.3 μL, 7.46 mmol) were added thereto. The resulting mixture was stirred overnight at room temperature in a nitrogen atmosphere. After the reaction, the solvent in the reaction solution was distilled off. The residue was filtrated using a KIRIYAMA funnel and washed with CH_2Cl_2 and H_2O. As a result, crude crystals of compound (11) were produced (3.0 mg, 73%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.33 (brs, 1H; NH), 10.34 (s, 1H; NH), 10.33 (s, 1H; NH), 10.27 (s, 1H; NH), 9.92 (s, 2H; NH), 9.73 (s, 1H; NH), 9.32 (brs, 1H; NH), 8.01 (brt, 1H; NH), 7.94 (s, 1H; CH), 7.56 (s, 1H; Im-H), 7.50 (s, 1H; Im-H), 7.46 (s, 1H; Im-H), 7.40 (brd, 1H, J = 8.5 Hz; CH), 7.31 (brd, 1H, J = 8.5 Hz; CH), 7.30 (d, 1H, J = 1.5 Hz; Py-H), 7.26 (d, 1H, J = 1.5 Hz; Py-H), 7.17 (d, 1H, J = 1.5 Hz; Py-H), 7.16 (s, 2H; y-Hx2), 6.90 (d, 1H, J = 1.5 Hz; Py-H), 6.85 (brs, 1H; CH), 4.00 (s, 3H; NCH_3), 3.97 (s, 3H; NCH_3), 3.95 (s, 3H; NCH_3), 3.85 (s, 3H; NCH_3), 3.84 (s, 3H; NCH_3), 3.80 (s, 3H; NCH_3), 3.20 (dt, 2H, J = 6.0 and 7.5 Hz; CH_2), 2.36 (t, 2H, J = 7.5 Hz; CH_2), 2.04 (s, 3H; $COCH_3$), 1.79 (qu, 2H, J = 7.5 Hz; CH_2);

ESI-TOFMS m/e calcd. for $C_{48}H_{51}N_{18}O_{10}$

$[M^+ + H]$ 1039.40, found 1039.39

[0047]

(4) Synthesis of AcImImPyPy-γ-ImPy-Indole-CBI (13)

Into a reaction vessel containing the crude crystals of compound (11) (3.0 mg, 2.89 mmol), seco-CBI (5) (1.4 mg, 6.01 mmol), EDCI (1.2 mg, 6.25 mmol),

and $NaHCO_3$ (1.0 mg, 12.0 mmol) were charged. The resulting mixture was dissolved in DMF (100 μL), and the resulting solution was stirred at room temperature for 2 hours in a nitrogen atmosphere. After confirmation of completion of the reaction, 5% $NaHCO_3$ (300 μL) and DMF (300 μL) were added thereto. The resulting mixture was stirred for 30 minutes. After completion of the reaction, the solvent was distilled off. The resulting residue was purified by silica-gel column chromatography (5% to 15% MeOH in CH_2Cl_2, gradient elution). The solvent was distilled off to yield crude crystals (13) (1.33 mg, 27% for 2 steps). Furthermore, purification was performed by HPLC (linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting solution was subjected to vacuum concentration and lyophilization to yield yellow crystals (13) (0.5 mg, 0.41 μmol; 11% for 2 steps).

^{1}H-NMR (500 MHz, DMSO-d_6) δ: 11.77 (s, 1H; NH), 10.32 (s, 1H; NH), 10.29 (s, 1H; NH), 10.26 (s, 1H; NH), 9.95 (s, 1H; NH), 9.92 (s, 1H; NH), 9.82 (s, 1H; NH), 9.33 (s, 1H; NH), 8.08 (s, 1H; CH), 8.01 (brs, 1H; NH), 8.00 (d, J = 7.5 Hz, 1H; CH), 7.60 (t, J = 7.5 Hz, 1H; CH), 7.56 (s, 1H; CH), 7.53 (d, J = 8.5 Hz, 1H; CH), 7.50 (s, 1H; CH), 7.46 (s, 1H; CH), 7.43 (t, J = 7.5 Hz, 1H; CH), 7.42 (s, 1H; CH), 7.31 (s, 1H; CH), 7.27 (s, 1H; CH), 7.24 (d, J = 8.5 Hz, 1H; CH), 7.22 (s, 1H; CH), 7.19 (s, 1H; CH), 7.16 (d, J = 7.5 Hz, 1H; CH), 7.06 (s, 1H; CH), 6.95 (s, 1H; CH), 6.89 (s, 1H; CH), 4.62 (dd, J = 10.0 and 5.0 Hz, 1H; NCH_2), 4.48 (d, J = 10.0 Hz, 1H; NCH_2), 4.00 (s, 3H; NCH_3), 3.97 (s, 3H; NCH_3), 3.95 (s, 3H; NCH_3), 3.86 (s, 3H; NCH_3), 3.84 (s, 3H; NCH_3), 3.80 (s, 3H; NCH_3), 3.20 (m, 2H; CH_2), 2.90 (m, 1H; CH), 2.35 (m, 2H; CH_2), 2.03 (s, 3H; $COCH_3$), 1.79 (m, 2H; CH_2), 1.76 (dd, J = 7.5 and 5.0 Hz, 1H; CH), 1.70 (t, J = 5.0 Hz, 1H; CH);

ESI-TOFMS m/e calcd. for $C_{61}H_{60}N_{19}O_{10}$

$[M^+ + H]$ 1218.48, found 1218.48

[0048]

(5) Synthesis of AcImImPy-γ-Im-Indole-CBI (14)

Compound (14) was synthesized in the same synthetic procedure as compound (13). Purification was performed by HPLC with a Chemcobond 5-ODS-H column (conditions: linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting compound was used for a DNA-alkylating reaction.

ESI-TOFMS m/e calcd. for $C_{49}H_{48}N_{15}O_8$

$[M^+ + H]$ 974.37, found 974.26

[0049]

(6) Synthesis of AcImImPyPy-γ-PyPy-Indole-CBI (15)

Compound (15) was synthesized in the same synthetic procedure as compound (13). Purification was performed by HPLC with a Chemcobond 5-ODS-H column (conditions: linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting compound was used for a DNA-alkylating reaction.

ESI MS m/e calcd. for $C_{62}H_{61}N_{18}O_{10}$

$[M^+ + H]$ 1217.5, found 1217.4

[0050]

(7) Synthesis of AcImPyPyPy-γ-ImPy-Indole-CBI (16)

Compound (16) was synthesized in the same synthetic procedure as compound (13). Purification was performed by HPLC with a Chemcobond 5-ODS-H column (conditions: linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting compound was used for a DNA-alkylating reaction.

ESI-TOFMS m/e calcd. for $C_{62}H_{61}N_{18}O_{10}$

$[M^+ + H]$ 1217.5, found 1217.4

[Embodiment 7]

Synthetic Reaction of Compound (17)

[0051]

Synthesis of AcImImPyPyβPyPy-γ-ImPyβImPy-Indole-CBI (17)

Compound (17) was synthesized in the same synthetic procedure as compound (13). Purification was performed by HPLC with a Chemcobond 5-ODS-H column (conditions: linear gradient of 0-50% acetonitrile in 0.1% acetic acid, 40 min, 254 nm). The resulting compound was used for a DNA-alkylating reaction.

ESI MS m/e calcd. for $C_{90}H_{93}N_{30}O_{16}$

$[M^+ + H]$ 1849.7, found 1850.1

Reagents, such as N,N-diisopropylethylamine ($^iPr_2NEt$) and O-(7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium (HATU), solvents, such as N,N-dimethylformamide (DMF), and the like were mainly purchased from a reagent manufacturer, such as Sigma-Aldrich Co., and used without purification. Unless otherwise specified, the reaction was monitored by high-performance liquid chromatography (HPLC) at a UV wavelength of 254nm UV. The ^1H-NMR spectrum was measured with JEOL JNM-A 500 (500 MHz) using tetramethylsilane (TMS) as an internal standard. With respect to multiplicity, a singlet, a doublet, a triplet, a quartet, a quintet, a multiplet, and broad were abbreviated as s, d, t, q, qu, m, and br, respectively. Electrospray ionization mass spectra (ESIMS) were measured with a PE SCIEX API 165. Electrospray ionization time-of-flight mass spectrometry (ESI-TOFMS) was performed on a BioTOFII (Bruker Daltonics) mass spectrometer. Polyacrylamide gel electrophoresis was performed on a HITACHI 5500-S DNA Sequencer. The loading dye (dimethylformamide with fushin red) was purchased from Amersham Co. Ltd., and 50% Long Ranger™ gel solution was purchased from FMC Bioproducts. Calf intestine alkaline phosphatase (AP, 1,000 unit/mL) was purchased from Boehringer Mannheim.

[Brief Description of Drawing]

[0052]

Fig. 1 shows abilities of compounds (6) and (7) of the present invention to DNA-sequence-specifically alkylate a long-chain DNA (pUC-II and pUC-II'); and a sequence recognition model thereof.

Fig. 2 shows the abilities of compounds (13) and (14) of the present invention to DNA-sequence-specifically alkylate a long-chain DNA (pUC-II and pUC-I') and sequence recognition models thereof.

Fig. 3 shows a model of base-sequence-specific alkylation of DNA by compound (13) of the present invention.

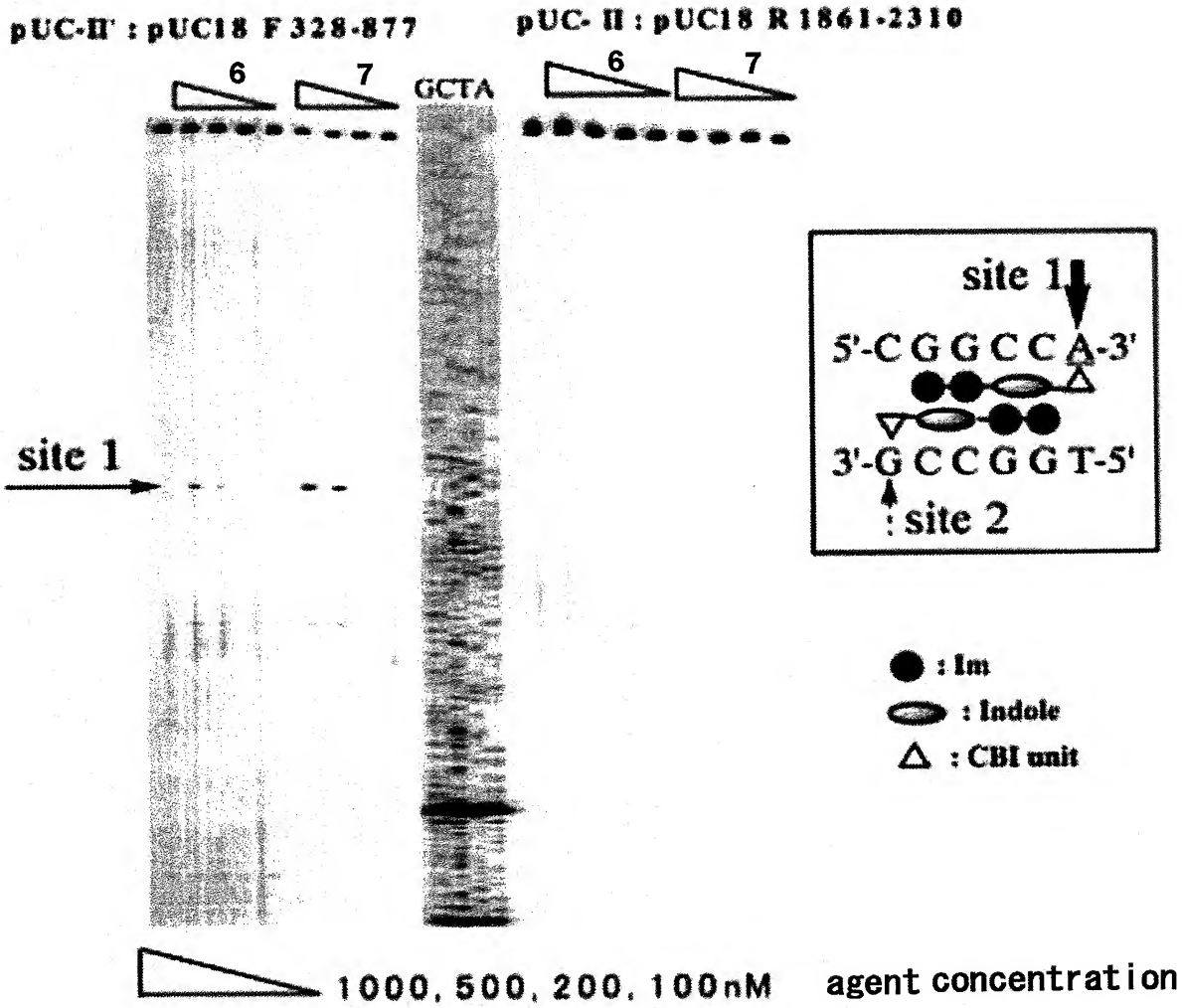
Fig. 4 shows abilities of compounds (13), (15), and (16) of the present invention to DNA-sequence-specifically alkylate a long-chain DNA (λ-F10906); and sequence recognition models thereof.

Fig. 5 shows the DNA-sequence-specific alkylation of a long-chain DNA (pQBI63) by compound (17) of the present invention.

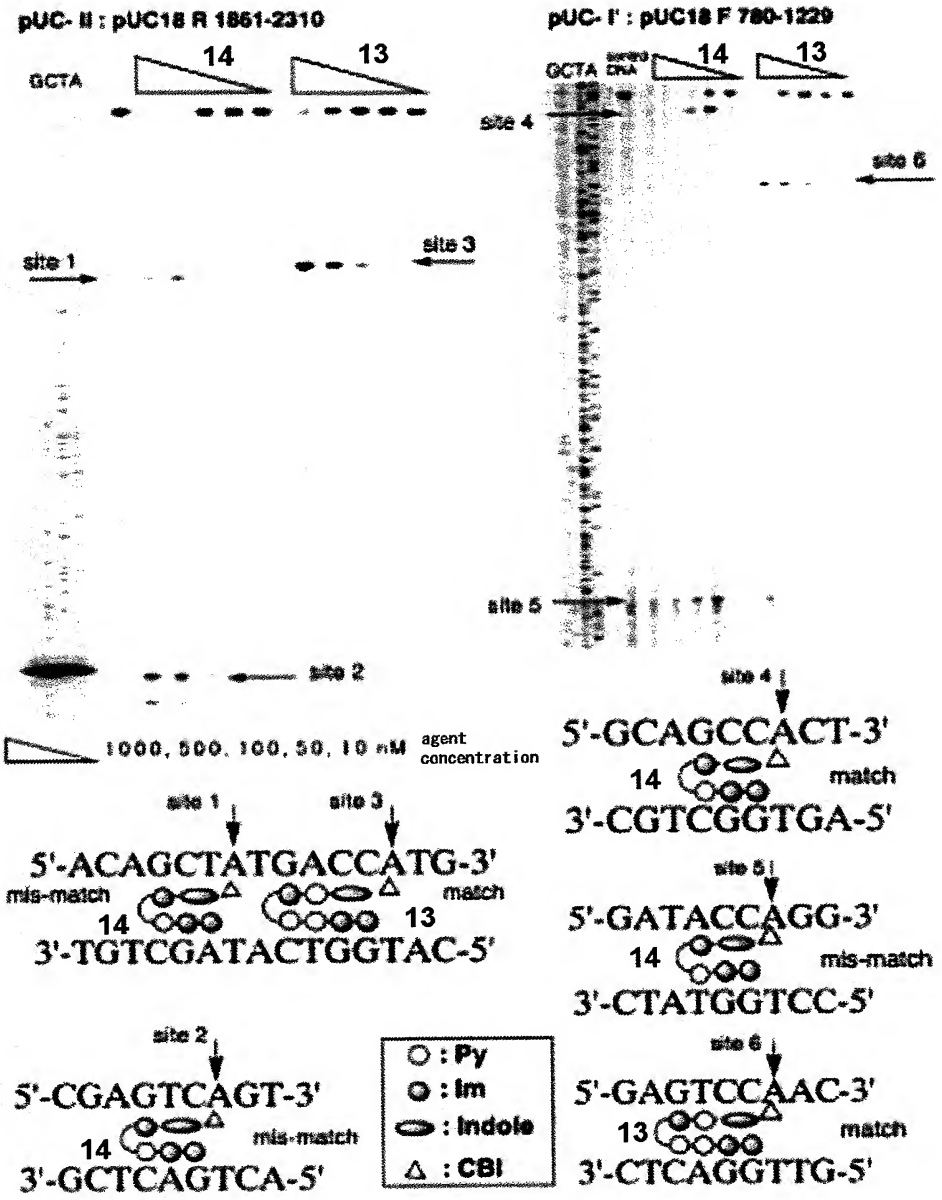
Fig. 6 shows stability of compounds (6) and (7) of the present invention under acidic and alkaline conditions.

【Document】 Drawings

【Fig.1】



【Fig.2】


pUC- II : pUC18 R 1861-2310
pUC- I' : pUC18 F 780-1229
GCTA
14
13
site 1
site 2
site 3
site 4
site 5
site 6
1000, 500, 100, 50, 10 nM agent concentration
5'-ACAGCTATGACCATG-3'
3'-TGTCGATACTGGTAC-5'
mis-match
match
5'-GCAGCCACT-3'
3'-CGTCGGTGA-5'
5'-GATACCAGG-3'
3'-CTATGGTCC-5'
5'-CGAGTCAGT-3'
3'-GCTCAGTCA-5'
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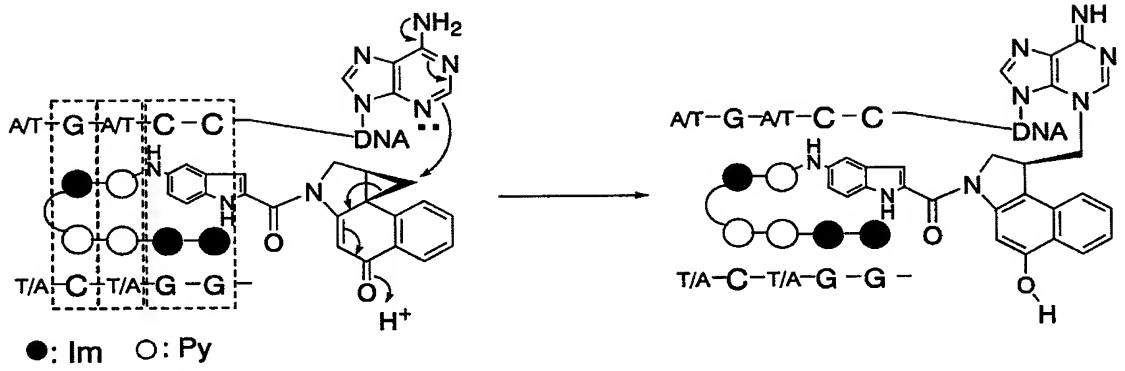
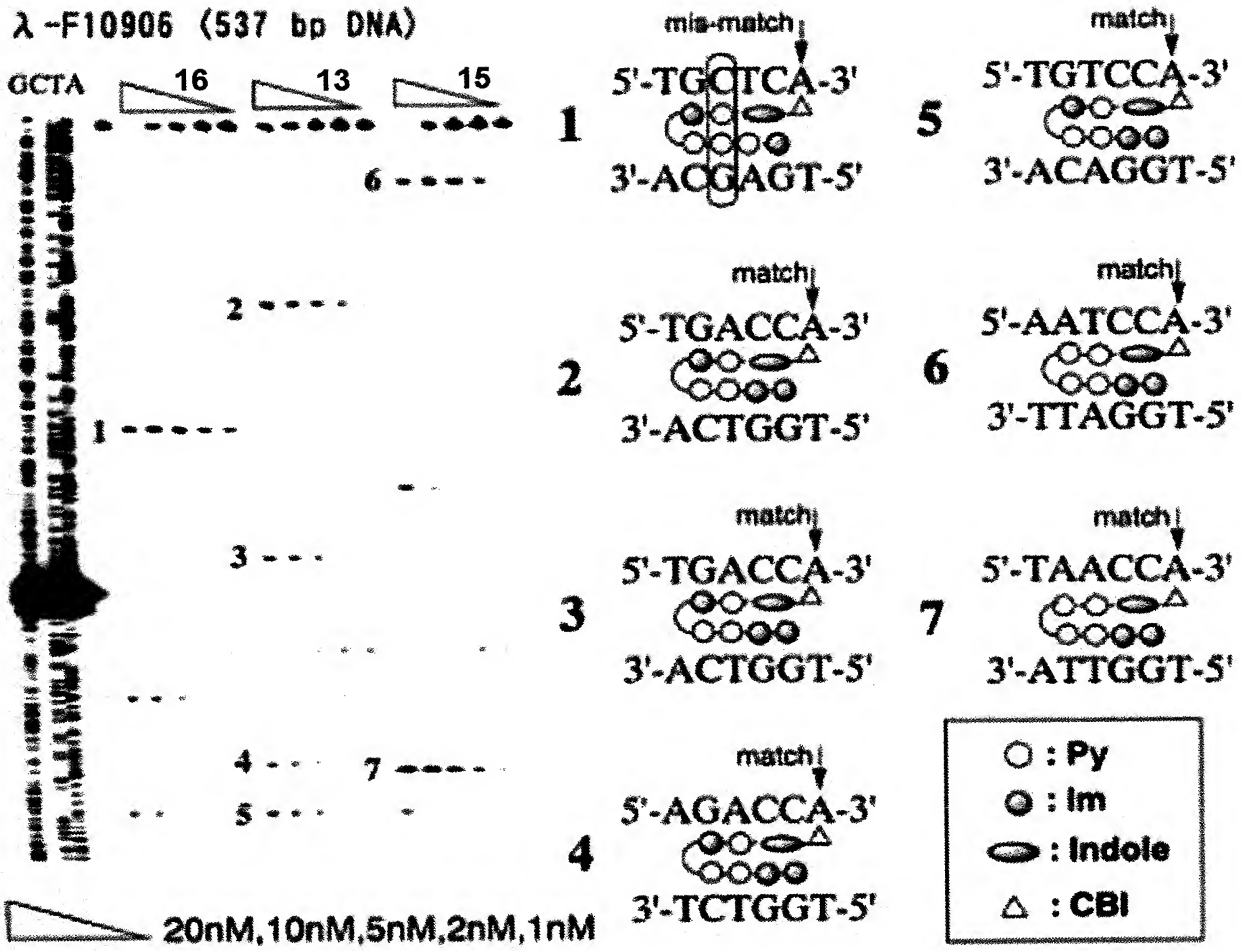
: Py
: Im
: Indole
: CBI

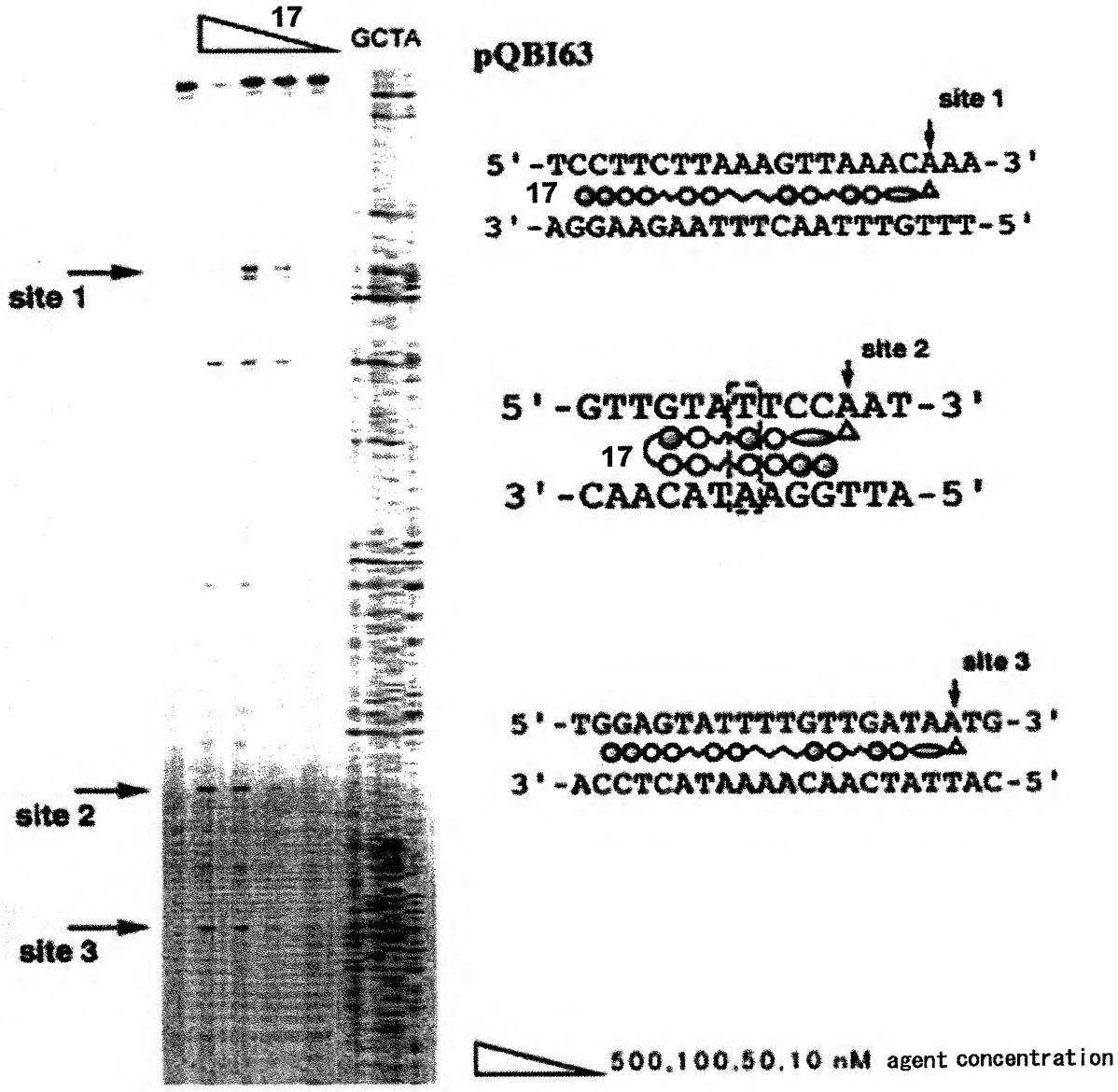
【Fig.3】


A/T-G-A/T-C-C
T/A-C-T/A-G-G-
DNA
H+
●: Im ○: Py

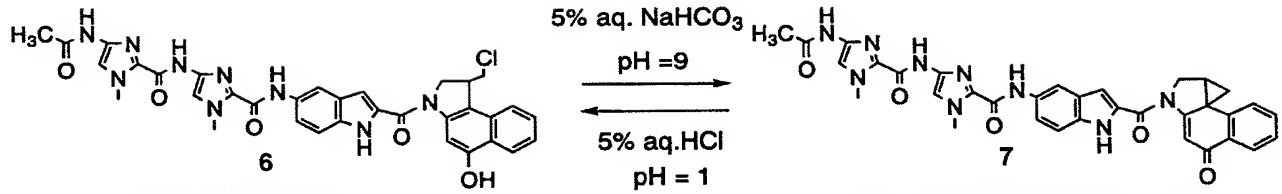
【Fig.4】


λ-F10906 (537 bp DNA)
GCTA
16
13
15
6
2
1
3
4
7
5
20nM,10nM,5nM,2nM,1nM
mis-match
5'-TGCTCA-3'
3'-ACGAGT-5'
1
match
5'-TGTCCA-3'
3'-ACAGGT-5'
5
match
5'-TGACCA-3'
3'-ACTGGT-5'
2
match
5'-AATCCA-3'
3'-TTAGGT-5'
6
match
5'-TGACCA-3'
3'-ACTGGT-5'
3
match
5'-TAACCA-3'
3'-ATTGGT-5'
7
match
5'-AGACCA-3'
3'-TCTGGT-5'
4
: Py
: Im
: Indole
: CBI

【Fig.5】


17
GCTA
pQBI63
site 1
5'-TCCTTCTTAAAGTTAAACAAA-3'
17
3'-AGGAAGAATTTCAATTTGTTT-5'
site 1
site 2
5'-GTTGTATTCCAAT-3'
17
3'-CAACATAAGGTTA-5'
site 2
site 3
5'-TGGAGTATTTTGTTGATAATG-3'
3'-ACCTCATAAAACAACTATTAC-5'
site 3
500,100,50,10 nM agent concentration

【Fig.6】


5% aq. NaHCO3
pH =9
5% aq.HCl
pH = 1
6
7
5% aq.HCl (pH =1)
not decomposed at 37℃
after 24 h (> 95% retained)
5% aq.NaHCO3 (pH = 9)
not decomposed at 37℃
after 24 h (> 95% retained)

[Document] Abstract

[Abstract]

[Problem]

Conventionally, the synthetic yield of alkylated hairpin polyamide is low and there is a problem in the chemical stability of vinyl linker important for alkylation reaction. Therefore, it has been impossible to adopt automatic syntheses using peptide synthesizer, and to synthesize alkylated hairpin polyamide corresponding to various base sequences.

[Means for solution]

The inventor have conducted intensive studies for linker having high alkylation ability and stability, and solved the problems by linking hairpin polyamide to alkylation moiety by using indole linker. The present invention precisely recognized base sequence of DNA and displayed an effect for cancer cells. Establishment of the synthetic pathway using combination of indole linker and solid phase synthesis made it possible to provide an alkylating function agent, thereby a possibility to create a novel anticancer agent that has the ability to recognize a gene sequence particular to cancer cells has arisen.